AUTOMATED QUANTIFICATION OF INTERSTITIAL AND PERIVASCULAR CARDIAC FIBROSIS
IN THE MREN2(27) TRANSGENIC RAT MODEL

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INTRODUCTION

Fibrosis is a naturally occurring process that results from the accumulation of collagen proteins in several tissues throughout the body. While these increases in collagen often occur with age, over accumulation resulting in fibrosis can be aggravated by disorders such as hypertension [1]. Hypertension is currently a prevalent issue in today’s society, and studies show that with this disorder, increased fibrosis can lead to damaging effects in cardiac tissue [2]. As collagen accumulates in ventricular tissues overall heart performance and morphology changes, similar to affects brought on by myocardial infarction or diseases such as diabetes [3-4]. Before treatment options can be explored for excess cardiac fibrosis, first researchers must confirm a solid model of fibrosis in the heart versus a control.

Previous studies indicate mRen2(27) transgenic rats as an example of increased hypertension [5]. These rats are designed to overexpress renin, which through activation of the renin-angiotensin system (RAS), subsequently exhibit several symptoms of increased cardiac fibrosis. The mechanisms by which renin causes downstream effects on cardiac fibrosis are due to increased angiotensin (II) linked to RAS activity. Increased angiotensin (II) is a known mediator of hypertension, and linked to cardiomyocyte hypertrophy, left ventricular (LV) dysfunction, and interstitial cardiac fibrosis. Changes exhibited by transgenic rats in cardiac tissue included left ventricular hypertrophy, increased sodium-proton exchange, overall heart size, and increased vascular thickness [6].

Previous studies have also explored this as a possible model, one indicating cardiac dysfunction exhibited by transgenic rats vs spontaneously hypertensive rats, supporting the hypothesis that increased hypertension does indeed exacerbate the effects of cardiac fibrosis [7]. While several methods exist to quantify collagen protein expression, the Picrosirius Red method is a proven, reliable method for specifying collagen protein in tissue. Due to its binding properties, the dye elongates itself along the parallel axis, and stained collagen will exhibit birefringence under polarized light filters [8].

OBJECTIVE

The aim of this study was to utilize Picrosirius Red staining to quantify myocardial collagen in the mRen2 transgenic rat and its sex- and age- matched Wistar-Han control (WHC). In conjunction with this previous knowledge on the transgenic rat model and the role of hypertension, the ultimate goal is to depict the heart on a functional, genetic, and protein level. Based on previous knowledge, a good animal model of cardiac fibrosis on the protein level should indicate increased levels of overall cardiac fibrosis in interstitial and perivascular tissue. Additionally this study aimed to optimize a quantification algorithm to assist in collagen content analysis. Through understanding of the protein expression of collagen in the tissue, this study will further the completion of a complete whole heart model of cardiac fibrosis.

METHODS

Samples of LV from both heterozygous mRen2 transgenic rats (n=4) and WHC rats (n=8) were isolated, weighed, and cut into two cross-sections. Each was stored overnight in 10% formalin solution and embedded in paraffin. These samples were sectioned at 5μm and mounted onto slides in preparation for histological staining. The slides were then deparaffined and washed with xylene before being rehydrated with a descending ethanol gradient. The samples were stained with picrosirius red dye (0.5% in saturated picric acid) for 80 minutes. Following the completion of the stain, slides were then dehydrated through an ascending ethanol gradient and mounted with a coverslip for examination via light microscope (Olympus Provis bright-field microscope). Bright-field images were collected at 4x magnification on four quadrants of each LV sample. Additionally, representative perivascular images were taken at 20x magnification.

The researcher taking the images was blinded to the identity of each sample. Olympus microscopes were set at a predetermined exposure time and transmitted light setting. ImageJ, a NIH approved image-editing software, was used to process and organize images according to tissue type prior to collagen assessment. Using a custom-developed automated algorithm, images were analyzed based on saturation thresholds that were calculated within bounds set by the algorithm, versus manually-set saturation thresholds. The four quadrants of each sample were combined and the ratio of collagen area to total tissue area was expressed as a percentage. Perivascular measurements were expressed as an average percentage of perivascular tissue area.

RESULTS

Transgenic rats showed a significant increase in interstitial collagen protein expression versus WHC rats, and a tendency for increase in perivascular collagen protein expression. mRen2 rats exhibited an average collagen to total tissue area of 3.95% ± 1.01% (n=4). This was overall a 67% increase in interstitial collagen content versus WHC rats, which exhibited a ratio of 1.96% ± 0.06% (n=7); \(P = 0.012\) (Figure 1A). Perivascular content showed a slight increase in collagen as a percentage of perivascular tissue area; however, this did not reach statistical significance (mRen2: 28.54% ± 6.65% vs. WHC: 23.05 ± 0.45%; \(P = 0.161\) (Figure 1B).

There was a significant linear correlation between the collagen content values obtained by the two methods, both for interstitial collagen (\(R^2 = 0.86\), Figure 1C) and perivascular collagen (\(R^2 = 0.74\), Figure 1D) data. There was a difference...
between the absolute values obtained by the two methods. The spearman’s rank-order correlation (\(r\)) was highly significant (\(P = 0.001\)) for perivascular collagen data (\(r = 0.84\), Figure 1D), indicating that there was significant strength and direction of association between the two methods. Similar associative strength was observed for intestinal collagen data (\(r = 0.56\), Figure 1C); however, the \(r\) value did not reach statistical significance (\(P = 0.07\)).

**DISCUSSION**

The mRen2 transgenic rat model, a proven hypertensive animal novel to fibrosis research, was compared with WHC sex- and age- matched controls. Previous papers have detailed the mRen2 rat as a model of disease versus other hypertensive or treated species. The purpose behind this study’s method was to compare a “sick” model of fibrosis with one whose collagen content accumulates naturally. Due to the downstream effects of the activated renin-angiotensin system, interstitial collagen levels in transgenic rats were significantly higher than WHC counterparts. Despite a slight difference in perivascular collagen content, overall change was not as significant. ImageJ has often proved useful to researchers as a tool to process and collect data from image based experiments. Further analysis of rank-order determined a strong association between the automated and manual assessments for perivascular collagen content, and to a lesser degree interstitial collagen content. With a clear change in interstitial collagen content, a future study into genetic and functional assessments would be the next reasonable step. The increased collagen may be the result of increased activity at the gene level, or is it the result of another system or protein? Additionally, do small physical differences indicate the possibility transgenic rats exhibit the same, better, or worse cardiac performance than the WHC rats? These are all future aspects of the total heart model of fibrosis that will combine with this studies assessment of protein expression in cardiac tissue. Previous studies have studied the function of mRen2 transgenic rats, however, did not include a control. Future implications of these findings will lead towards an investigation into possible treatments for increased cardiac fibrosis.

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**REFERENCES**


